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Studies on the Constituents of Asclepiadaceae Plants. LXV.[1] The Optical Resolution of D- and L-Cymaroses

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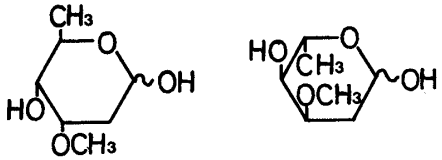
The carbamoyl derivatives of enantiomeric mixtures of methyl α- and β-cymaropyranosides were resolved by high-performance liquid chromatography (HPLC).

Keywords——optical resolution; D-cymarose; L-cymarose; HPLC; Asclepiadaceae; *Cynanchum wilfordi*; *Cynanchum africanum*; 3,5-dinitrophenyl carbamate

In the previous papers we reported the structures of glycosides isolated from *Cynanchum wilfordi* HEMSLEY[2] and *Cynanchum africanum* R. BR.[1,3] Their structures were unusual in that they include both D- and L-cymaroses in the sugar chain. The numbers of D- and L-cymaroses in the glycoside were deduced on the basis of both the optical rotation of cymarose obtained from the acidic hydrolysate of the glycoside and the analysis of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of the glycoside. A considerable amount of glycoside (60—100 mg) was required to measure the optical rotation of cymarose, however. In this paper we wish to describe the optical resolution of D- and L-cymaroses achieved by high-performance liquid chromatography (HPLC) with a few mg of sample.



D-cymarose L-cymarose

Chart 1

Four samples of cymarose were used for the experiment: D-cymarose derived from *C. caudatum* MAX.,[4] $[\alpha]_D^{15}$ +51.6° (c=1.02, H_2O); L-cymarose derived from *C. glaucescens* HAND-MAZZ,[5] $[\alpha]_D^{13}$ −50.4° (c=1.00, H_2O); an enantiomeric mixture (an excess of D-enantiomer) from *C. wilfordi*, $[\alpha]_D^{20}$ +24.5° (c=1.34, H_2O); another mixture (an excess of L-enantiomer) from *C. atratum* BUNGE,[6] $[\alpha]_D^{15}$ −29.4° (c=1.04, H_2O). Methylglycosylation of cymarose usually afforded a mixture of methyl α- and β-cymaropyranosides and methyl α- and β-cymarofuranosides in the ratio of 1 : 3 : 10 : 5, respectively, on the basis of the intensity of 6-CH_3 signals in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Each mixture of methyl cymarosides derived from the four samples was allowed to react with 3,5-dinitrophenyl isocyanate in dry toluene in the presence of dry pyridine to give the carbamates, which were analyzed by HPLC (Fig. 1). On the other hand, the mixture of methyl cymarosides from *C. wilfordi* was separated into each enantiomeric mixture of methyl α- (**3**) and β-

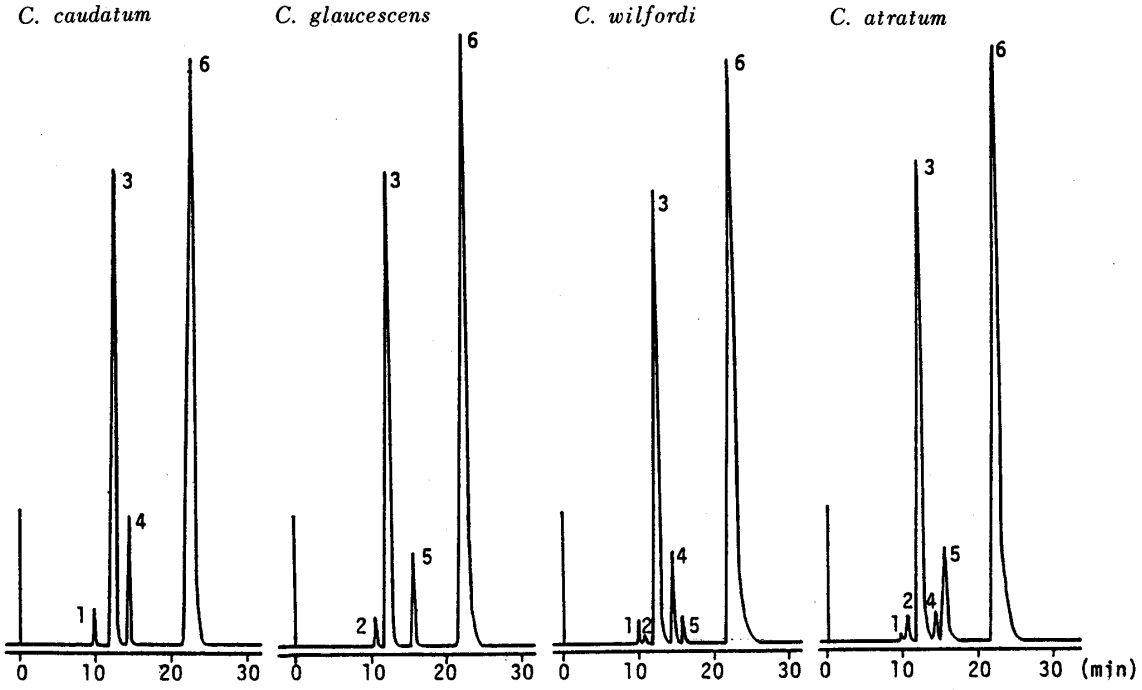


Fig. 1. High-Performance Liquid Chromatogram of Carbamates of Methyl Cymarosides Derived from *C. caudatum, C. glaucescens, C. wilfordi,* and *C. atratum*

Conditions: Column, SUMIPAX OA-1000 (5 μ, 4 mm i.d. × 15 cm); mobile phase, *n*-hexane–1,2-dichloroethane–ethanol (30 : 6 : 1), flow rate, 1.0 ml/min; detector, UV (254 nm).
Peak 1, methyl α-D-cymaropyranoside 3,5-dinitrophenylcarbamate (**9**); Peak 2, methyl α-L-cymaropyranoside 3,5-dinitrophenylcarbamate (**11**); Peak 3, methyl β-DL-cymarofuranoside 3,5-dinitrophenylcarbamate (**14**); Peak 4, methyl β-D-cymaropyranoside 3,5-dinitrophenylcarbamate (**10**); Peak 5, methyl β-L-cymaropyranoside 3,5-dinitrophenylcarbamate (**12**); Peak 6, methyl α-DL-cymarofuranoside 3,5-dinitrophenylcarbamate (**13**).

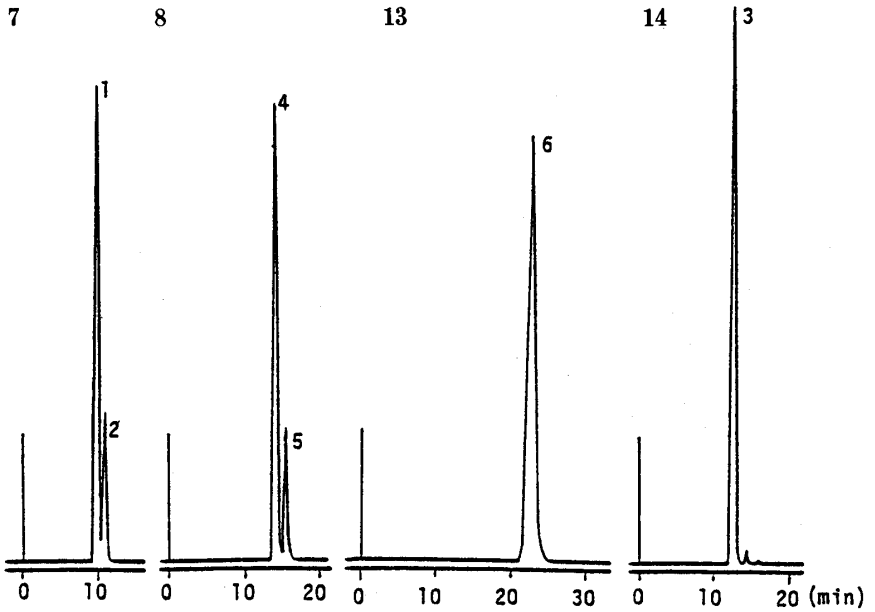


Fig. 2. High-Performance Liquid Chromatogram of Carbamates **7**, **8**, **13**, and **14**

Conditions and abbreviations were the same as in Fig. 1.

cymaropyranosides (**4**) and methyl α- (**5**) and β-cymarofuranosides (**6**), which were led to the carbamates. The HPLC results (Fig. 2) showed that methyl α-(**7**) and β-cymaropyranoside 3,5-dinitrophenylcarbamates (**8**) were resolved into the D-enantiomer (**9** for α-anomer, peak 1; **10** for β-anomer, peak 2) and the L-enantiomer (**11** for α-anomer, peak 4; **12** for β-anomer, peak 5). The difference of the peak intensities of D- and L-enantiomers (ratio of 3 : 1) was consist with the optical rotation of the cymarose derived from *C. wilfordi*. Methyl α- (**13**, peak 6) and β-cymarofuranosides 3,5-dinitrophenylcarbamates (**14**, peak 3), however, were not optically resolved under the present conditions. The molecular formulae of **7**, **8**, **13**, and **14** ($C_{15}H_{10}N_3O_9$) were confirmed by elemental analysis and field-desorption mass spectra (FD-MS). Their infrared (IR) spectra lacked hydroxyl absorption bands but showed 3,5-dinitrophenylcarbamoyl absorption bands at 3420, 1740, 1605, 1550, 1530, 1345, 1240, 1110, 800—700, and 660 cm^{-1}. Owing to the carbamoylation, the signals of 4-CH of **3** and **4** were shifted from δ 3.25 and 3.23 to δ 4.72 and 4.69, respectively. Signals of 5-CH of **5** and **6** were shifted from δ 4.00 and 3.92 to δ 5.11 and 5.13, respectively. Judging from the intensities of peaks 1, 2, 4, and 5 (Fig. 1), cymarose derived from *C. caudatum* consists of only the D-enantiomer, and cymarose from *C. glaucescens* consists of only the L-enantiomer, while cymarose from *C. atratum* is composed of an enantiomeric mixture (an excess of the L-enantiomer).

Many of the glycosides from Asclepiadaceae plants have D- and/or L-cymaroses in the sugar chain. The method described in this paper defined the ratio of D- and L-cymaroses unequivocally. It is available for the structural elucidation of a small amount of glycoside.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ with a JASCO DIP-4 digital polarimeter at room temperature. Ultraviolet (UV) spectra were obtained in ethanol with a Hitachi 228A spectrometer, and absorption maxima are given in nm. IR spectra were recorded in $CHCl_3$ on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on a JEOL GX-270 (270.05 MHz) machine in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. FD-MS was carried out with a JEOL JMS-01SG-2. HPLC was conducted with a Waters 204 compact model, using a column of SUMIPAX OA-1000 (5 μ, 4 mm i.d. × 15 cm) (Nishio Industry Co., Ltd.) with *n*-hexane–1,2-dichloroethane–ethanol (30 : 6 : 1) as a mobile phase (flow rate, 1.0 ml/min; detector, UV (254 nm)). Thin layer chromatography (TLC) was performed on Merck precoated plates (Kiesel gel F_{254}) with the following solvent systems: Rf_1 *n*-hexane–ethyl acetate (1 : 4), Rf_2 *n*-hexane–ethyl acetate (3 : 2), and Rf_3 *n*-hexane–1,2-dichloroethane–ethanol (12 : 4 : 1). Column chromatography was carried out on Wakogel C-200 (200 mesh).

Methylglycosylation of Cymarose (1) Derived from the Crude Glycoside of *C. wilfordi*.——Isolation of **1** was described in the previous paper.[2a] A solution of **1** (2.48 g) in MeOH (150 ml) was allowed to react with 1% H_2SO_4–MeOH (150 ml) at room temperature for 30 min, then H_2O (150 ml) was added and the reaction mixture was neutralized with satd. $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a mixture of methyl cymarosides (**2**, 1.65 g). The product **2** was chromatographed on silica gel using ethyl acetate–*n*-hexane (1 : 4) to give methyl α-cymaropyranoside (**3**, 44.5 mg), methyl β-cymaropyranoside (**4**, 106.7 mg), methyl α-cymarofuranoside (**5**, 699.1 mg), and methyl β-cymarofuranoside (**6**, 218.8 mg).

3: A colorless syrup. Rf_1 0.38. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.28 (3H, d, J=6.2 Hz, 6-CH_3), 1.75 (1H, ddd, J=15.0, 4.8, 3.7 Hz, 2-CH_{ax}), 2.27 (1H, ddd, J=15.0, 3.3, 1.5 Hz, 2-CH_{eq}), 2.52 (1H, d, J=10.6 Hz, 4-COH), 3.25 (1H, ddd, J=10.6, 9.5, 3.7 Hz, 4-CH), 3.35, 3.44 (each 3H, s, 1,3-OCH_3), 3.59 (1H, dt, J=3.3, 3.7 Hz, 3-CH), 3.85 (1H, dq, J=9.5, 6.2 Hz, 5-CH), 4.65 (1H, dd, J=4.8, 1.5 Hz, 1-CH).

4: A colorless syrup. Rf_1 0.50. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.31 (3H, d, J=6.2 Hz, 6-CH_3), 1.56 (1H, ddd, J=14.3, 9.5, 2.9 Hz, 2-CH_{ax}), 2.26 (1H, ddd, J=14.3, 3.7, 2.2 Hz, 2-CH_{eq}), 2.29 (1H, d, J=10.6 Hz, 4-COH), 3.23 (1H, ddd, J=10.6, 9.5, 3.7 Hz, 4-CH), 3.43, 3.48 (each 3H, s, 1,3-OCH_3), 3.61 (1H, dq, J=9.5, 6.2 Hz, 5-CH), 3.63 (1H, dt, J=2.9, 3.7 Hz, 3-CH), 4.57 (1H, dd, J=9.5, 2.2 Hz, 1-CH).

5: A colorless syrup. Rf_1 0.37. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.23 (3H, d, J=6.6 Hz, 6-CH_3), 2.05 (2H, m, 2-CH_2), 3.34, 3.39 (each 3H, s, 1,3-OCH_3), 3.90 (1H, m, 3-CH), 3.92 (1H, t, J=2.9 Hz, 4-CH), 4.00 (1H, dq, J=2.9, 6.6 Hz, 5-CH), 5.07 (1H, dd, J=4.8, 1.8 Hz, 1-CH).

6: A colorless syrup. Rf_1 0.48. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.22 (3H, d, J=6.6 Hz, 6-CH_3), 2.15 (1H, ddd, J=13.9, 5.5, 4.4 Hz, 2-CH), 2.23 (1H, ddd, J=13.9, 7.0, 2.2 Hz, 2-CH), 3.30, 3.41 (each 3H, s, 1,3-OCH_3), 3.92 (1H,

dq, J=6.6, 2.6 Hz, 5-CH), 3.97 (1H, t, J=2.6 Hz, 4-CH), 4.14 (1H, ddd, J=7.0, 4.4, 2.6 Hz, 3-CH), 5.12 (1H, dd, J=5.5, 2.2 Hz, 1-CH).

Carbamoylation of 2, 3, 4, 5, and 6——A solution of **2** (5.4 mg) in dry toluene (0.5 ml) was allowed to react with 3,5-dinitrophenyl isocyanate (*ca.* 10 mg) in the presence of dry pyridine (0.05 ml) at 60 °C for 30 min, then the solvent was evaporated off. Carbamoylations of **3** (44.5 mg), **4** (44.4 mg), **5** (23.8 mg), and **6** (19.8 mg) were carried out, in the same way as described above. The products were chromatographed on silica gel using *n*-hexane–ethyl acetate (6 : 1) to afford carbamates **7** (94.2 mg), **8** (91.4 mg), **13** (48.3 mg), and **14** (32.9 mg), each of which crystallized as needles (**7**, 55.0 mg; **8**, 61.8 mg; **13**, 24.4 mg; **14**, 24.0 mg) from *n*-hexane–ethyl acetate. Carbamates **7** and **8** were separated into the D-enantiomer (**9**, 21.9 mg; **10**, 15.2 mg) and the L-enantiomer (**11**, 8.7 mg; **12**, 7.2 mg) by HPLC.

An Enantiomeric Mixture of Methyl α-Cymaropyranoside 3,5-Dinitrophenylcarbamate (**7**): Colorless needles. Rf_2 0.28, Rf_3 0.48. mp 180—182 °C. *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.90; H, 5.10; N, 10.72. FD-MS *m/z*: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 227 (4.64), 248 (4.37), 343 (3.54). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1470, 1450, 1345, 1240, 1110, 800—700, 660. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.30 (3H, d, J=6.2 Hz, 6-CH_3), 2.03 (1H, dt, J=15.0, 4.0 Hz, 2-CH_{ax}), 2.30 (1H, ddd, J=15.0, 4.0, 1.8 Hz, 2-CH_{eq}), 3.27, 3.42 (each 3H, s, 1,3-OCH_3), 3.94 (1H, dt, J=3.7, 4.0 Hz, 3-CH), 4.26 (1H, dq, J=9.2, 6.2 Hz, 5-CH), 4.72 (1H, dd, J=9.2, 3.7 Hz, 4-CH), 4.78 (1H, dd, J=4.0, 1.8 Hz, 1-CH), 8.71 (1H, t, J=2.0 Hz, *p*-aromatic H), 8.82 (2H, d, J=2.0 Hz, *o*-aromatic H).

Methyl α-D-Cymaropyranoside 3,5-Dinitrophenylcarbamate (**9**): $[\alpha]_D^{18}$ +161° (c=1.00, $CHCl_3$).

Methyl α-L-Cymaropyranoside 3,5-Dinitrophenylcarbamate (**11**): $[\alpha]_D^{18}$ −152° (c=0.70, $CHCl_3$).

An Enantiomeric Mixture of Methyl β-Cymaropyranoside 3,5-Dinitrophenylcarbamate (**8**): Colorless needles. Rf_2 0.42, Rf_3 0.43. mp 146—148 °C. *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.87; H, 4.92; N, 10.63. FD-MS *m/z*: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 226 (4.55), 247 (4.26), 343 (3.41). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1740, 1605, 1550, 1530, 1470, 1450, 1345, 1240, 1100, 800—700, 660. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.32 (3H, d, J=6.2 Hz, 6-CH_3), 1.75 (1H, ddd, J=13.9, 8.4, 2.9 Hz, 2-CH_{ax}), 2.25 (1H, ddd, J=13.9, 5.1, 2.2 Hz, 2-CH_{eq}), 3.43, 3.49 (each 3H, s, 1,3-OCH_3), 3.92 (1H, dt, J=5.1, 2.9 Hz, 3-CH), 4.07 (1H, dq, J=8.4, 6.2 Hz, 6-CH), 4.69 (1H, dd, J=8.4, 2.9 Hz, 4-CH), 4.73 (1H, dd, J=8.4, 2.2 Hz, 1-CH), 8.66 (2H, d, J=2.2 Hz, *o*-aromatic H), 8.73 (1H, t, J=2.2 Hz, *p*-aromatic H).

Methyl β-D-Cymaropyranoside 3,5-Dinitrophenylcarbamate (**10**): $[\alpha]_D^{18}$ +3.2° (c=1.00, $CHCl_3$).

Methyl β-L-Cymaropyranoside 3,5-Dinitrophenylcarbamate (**12**): $[\alpha]_D^{18}$ −2.9° (c=0.84, $CHCl_3$).

An Enantiomeric Mixture of Methyl α-Cymarofuranoside 3,5-Dinitrophenylcarbamate (**13**): Colorless needles. Rf_2 0.28, Rf_3 0.37. mp 137—138 °C. *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.66; H, 5.00; N, 10.61. FD-MS *m/z*: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 226 (4.51), 247 (4.24), 343 (3.45). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1470, 1450, 1345, 1240, 1110, 800—700, 660. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.40 (3H, d, J=6.6 Hz, 6-CH_3), 2.08 (1H, ddd, J=13.2, 6.6, 5.5 Hz, 2-CH), 2.29 (1H, ddd, J=13.2, 6.6, 1.8 Hz, 2-CH), 3.31, 3.34 (each 3H, s, 1,3-OCH_3), 3.97 (1H, t, J=4.8 Hz, 4-CH), 4.08 (1H, dt, J=4.8, 6.6 Hz, 3-CH), 5.08 (1H, dd, J=5.5, 1.8 Hz, 1-CH), 5.11 (1H, dq, J=4.8, 6.6 Hz, 5-CH), 8.67 (2H, d, J=2.0 Hz, *o*-aromatic H), 8.72 (1H, t, J=2.0 Hz, *p*-aromatic H).

An Enantiomeric Mixture of Methyl β-Cymarofuranoside 3,5-Dinitrophenylcarbamate (**14**): Colorless needles. Rf_2 0.40, Rf_3 0.49. mp 112—114 °C. *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.89; H, 5.06; N, 10.91. FD-MS *m/z*: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 226 (4.62), 248 (4.34), 334 (3.52). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1470, 1450, 1345, 1240, 1100, 800—700, 660. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.38 (3H, d, J=6.6 Hz, 6-CH_3), 2.07 (1H, ddd, J=13.8, 2.9, 1.5 Hz, 2-CH), 2.15 (1H, ddd, J=13.8, 7.0, 4.8 Hz, 2-CH), 3.35, 3.39 (each 3H, s, 1,3-OCH_3), 3.83 (1H, ddd, J=7.0, 4.0, 2.9 Hz, 3-CH), 4.11 (1H, t, J=4.0 Hz, 4-CH), 5.09 (1H, dd, J=4.8, 1.5 Hz, 1-CH), 5.13 (1H, dq, J=4.0, 6.6 Hz, 5-CH), 8.67 (2H, d, J=2.0 Hz, *o*-aromatic H), 8.72 (1H, t, J=2.0 Hz, *p*-aromatic H).

Methylglycosylation and Carbamoylation of Cymaroses Derived from *C. caudatum*, *C. glaucescens*, and *C. atratum*——D-Cymarose (30.2 mg) derived from *C. caudatum* was methylglycosylated, and 6.6 mg of the product was carbamated. L-Cymarose (5.3 mg) derived from *C. glaucescens* was methylglycosylated, followed by carbamoylation. In the case of *C. atratum*, 4.0 mg of cymarose was methylglycosylated and carbamoylated in the same way as described above.

References and Notes

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